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Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences

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The chemokine receptor gene, *CCR5*, has become a central theme in studies of host genetic effects on HIV-1 pathogenesis ever since the discovery that the CCR5 molecule serves as a major cell surface co-receptor for the virus. A growing number of genetic variants within the coding and 5' regulatory region of *CCR5* have been identified, several of which have functional consequences for HIV-1 pathogenesis. Here we review the CCR5 literature describing CCR5 polymorphism and the functional ramifications that several of these variants have on HIV-1 infection and progression to AIDS. The multiplicity of *CCR5* genetic effects on HIV-1 disease underscores the critical importance of this gene in controlling AIDS pathogenesis and provides the logic for development of therapeutic strategies that target the interaction of HIV-1 envelope and CCR5 in HIV-1 associated disease.

INTRODUCTION

Chemokine receptors are cell surface molecules that bind peptide ligands called chemokines, thereby inducing migration of the receptor-bearing cells toward injured tissues that secrete chemokines into the bloodstream. It is through this mechanism that leukocytes are recruited into sites of inflammation (1,2). A number of host cell surface molecules are used by infectious agents to gain entry into cells and it is now clear that HIV-1 enters cells through an interaction involving chemokine receptors (3–7). So-called 'R5' isolates of HIV-1 use the chemokine receptor CCR5 to infect macrophages and primary T cells, and these isolates are present early after seroconversion indicating their role in initiation of HIV-1 infection (8–12). The eventual use of CXCR4 by isolates of HIV-1 (X4 isolates, which infect primary T cells and T cell lines) closely corresponds to the onset of AIDS, although R5 isolates do persist throughout the entire course of infection (8–12). The central role of CCR5 in HIV-1 infection demands a clear understanding of the relationship between this receptor and virus, including the genetic alterations of the *CCR5* gene that play a role in controlling infection and disease progression.

VARIATION IN THE CCR5 CODING REGION

CCR5-Δ32

The *CCR5* gene has been mapped to the short arm of chromosome 3 amongst a group of genes that encode multiple chemokine receptors (13). Soon after CCR5 was shown to behave as a co-receptor along with CD4 for HIV-1, the mutant allele *CCR5-Δ32*, which is characterized by a 32 bp deletion in the single coding exon of the gene, was identified in Caucasians (14–16). *CCR5-Δ32* does not produce a functional protein (15), explaining the near-complete protection against HIV-1 infection in individuals homozygous for the allele. Rare cases of HIV-1 infection in the absence of CCR5 have been reported (17–19), however, suggesting that X4 isolates can sometimes initiate HIV-1 infection. Individuals homozygous for *CCR5-Δ32* display no clinical symptoms and appear to be healthy. Other homologous chemokine receptors bind an overlapping set of chemokine ligands and may compensate for the absence of CCR5 in individuals homozygous for *CCR5-Δ32* (2).

Another effect of *CCR5-Δ32* includes slower progression to AIDS (by 2–4 years on average) after HIV-1 seroconversion in individuals heterozygous for the mutation (14,20–22). The frequency of the *CCR5-Δ32/+* genotype is ~20% in Caucasians, so this genotype has a significant population effect on progression to AIDS. The *CCR5-Δ32/+* genotype results in markedly diminished levels of CCR5 on the cell surface and low expression of CCR5 correlates with reduced infection of T cells by R5 isolates of HIV-1 *in vitro* (23). Rather than simply a gene dosage effect, formation of CCR5-Δ32/CCR5 heterocomplexes causes CCR5 to be retained in the endoplasmic reticulum resulting in reduced cell surface expression of the wild-type molecule (24). The *CCR5-Δ32/+* genotype is also associated with protection from AIDS-related lymphoma, a non-Hodgkin's B cell malignancy (25,26). Although the mechanism for this protection is not clear, B cells do express CCR5 on their cell surfaces, and RANTES, one of four chemokine ligands of CCR5, is mitogenic for B cells (26). It is possible that RANTES may play a role in lymphoma expansion via CCR5 before immune surveillance has a chance to eliminate the malignant cells. If so, then diminished levels of

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Table 1. Genetic variations of the *CCR5* gene

Variant	Nucleic acid substitution	Frequency		Reference
		Caucasians	African Americans	
I12T[a]	A25C	<1%	0	31
C20S[a]	T58A	<1%	0	31
A29S[a]	G85T	NT	1.5%	31
I42F[a]	A124T	<1%	NT	31
L55Q[a]	T164A	3–4%	<1%	30,31
R60S	G180T	NT	1%	31
S63C	A187T	<1%	0	unpublished data
A73V[a]	C218T	<1%	0	31
S75S	T215C	0	1.3%	31
C101X	T303A	NT	<1%	31
I164I	C492A	<1%	NT	31
Δ32 (185)	D32	10.0%	1.9%	
L215S	C664T	0	<1%	30
R223Q[b]	G668A	1.6%	<1%	30,31
228delK	680del3	<1%	0	31
299 (FS)[b]	893delC	0	0	30
V300V	C900A	<1%	0	31
G301V	G902T	<1%	0	31
R319H	G956A	<1%	0	unpublished data
P332P	C996T	0	<1%	30
A335V	C1004T	<1%	2.5%	30,31
Y339F	A1016T	0	2.6%	30,31

NT, not tested.
[a]Alleles tested for CCR5 ligand signaling (34); see text.
[b]Found at 3–5% in Asian populations (30).

CCR5 in *CCR5-Δ32/+* heterozygotes may be advantageous by indirectly controlling B cell expansion.

Additional coding region variants of the *CCR5* gene

The *CCR5-Δ32* mutation is estimated to have occurred ~700–2000 years ago (27,28) and since that time has increased to a frequency of 13% in some Northern European populations (27,29). It is absent in Africans and most Asian populations, and its frequency in African Americans (f = 0.02) can be explained by admixture. The rapid increase in frequency of this mutation over a relatively short period of time suggests that the *CCR5-Δ32* mutation has been subject to positive selection. If indeed the *CCR5-Δ32* mutation has been (and perhaps still is) under selective pressures, then it is likely that other mutations resulting in severe functional alterations will also be subject to selection. Twenty-one additional alleles of the *CCR5* coding region have been described (30,31), two of which we identified recently and have not been published previously (S63C and R319H). Mutations defining two of the alleles cause premature termination of translation (C101X and 893delC; Table 1). Frequencies of the variants were 0.04 or lower and, among patients screened, no samples were homozygous for any of these variants. Thus, the epidemiological consequence of these variants on HIV-1 infection or progression to AIDS could not be evaluated. Among the 22 total variants of the *CCR5* gene, however, 16 are protein altering (non-synonymous) and only four are synonymous variants. The high predominance of codon-altering variants (18/22 or 82%) is consistent with an adaptive accumulation of function-altering *CCR5* alleles (32).

Each of six variants (I12T, C20S, I42F, L55Q, A73V and C101X) in cohorts studied were identified in individuals carrying the *CCR5-Δ32* allele on the opposite haplotype (31,33). Only the single type of CCR5 variant molecule is expressed on the cell surfaces of these individuals since *CCR5-Δ32* does not encode a cell surface molecule. Individuals expressing C20S, I42F or C101X were negative for HIV-1, in spite of high risk for having been exposed to the virus. Individuals expressing I12T, A73V, and L55Q were infected with HIV-1, although in the case of I12T, it is possible that a chemokine receptor other than CCR5 was used to gain entry to the cell (see below). Whereas it was observed that three CCR5 variants heterozygous for *CCR5-Δ32* avoided HIV-1 infection after likely exposure, influence of these variants on HIV-1 infection would be required to conclude that they are protective.

Recently, we analyzed ligand signaling plus influence on HIV-1 infection for six of the naturally occurring variants (indicated by '[a]' in Table 1) located within the N-terminal third of the *CCR5* gene, including I42F and C20S (34). Binding of the normal CCR5 ligands (RANTES, MIP-1α and MIP-1β) to variants with substitutions in the first extracellular domain (I12T, C20S and A29S) was either completely abrogated or severely reduced, corresponding with an inability of these variants to transduce a chemotactic signal to requisite ligands. The I12T and C20S variants were also unable to function as co-receptors for R5 isolates of HIV-1, suggesting that these variants radically alter conformation of the CCR5 molecule. Since I12T was originally identified in an HIV-1-infected individual who was heterozygous for *CCR5-Δ32*, it is possible that the individual with I12T/CCR5-Δ32 was not resistant to R5 tropic HIV-1 infection. Alternatively, this individual may have become infected by an X4 isolate of HIV-1 using CXCR4 as a portal of entry (17–19). A29S supported HIV-1 infection *in vitro*, but failed to bind chemokine ligands efficiently. The phenotypic effects of variants located in the first and second transmembrane domains (I42F, L55Q and A73V) were quite different from those in the first extracellular domain, having 4–8-fold enhanced affinity for chemokine. These variants transduced a chemotactic response to RANTES, MIP-1α and MIP-1β, but they did not exhibit the typical attenuation of response at high ligand concentrations, perhaps resulting from their strong affinity for the chemokine ligands. Cells expressing all three transmembrane variants supported HIV-1 infection.

VARIATION IN THE PROMOTER REGION OF CCR5

Variable expression of *CCR5*

Regulation of CCR5 expression is likely to be complex, but an understanding of mechanisms controlling its expression could be of significant therapeutic value in AIDS prevention (35–39). Expression of CCR5 is restricted to activated and memory T cells, monocytes/macrophages, microglial cells (40–43) and, to a lesser extent, B cells (26). CCR5 cell surface expression is highly variable even in individuals who are homozygous for the normal allele of the gene (*CCR5-+/+*) (23). While it is not clear whether heterogeneity in protein expression correlates with differences in HIV-1 infection *in vivo*, peripheral blood mononuclear cells from individuals with the genotype *CCR5-+/Δ32* are not as easily infected with R5 isolates of HIV-1 *in vitro* as are individuals with *CCR5-+/+* (15). Individuals with the genotype *CCR5-+/Δ32* express lower levels of CCR5 correlating with relatively low viral loads (44) and slow progression to AIDS (14,20–22). Such observations have stimulated studies of the upstream promoter region of the *CCR5* gene that have addressed functional, genetic and epidemiological aspects of the region.

Characterization of the *CCR5* promoter

The original partial length genomic clone of *CCR5* showed that it specified a single open reading frame (ORF) (45). Comparison of this sequence with two cDNA clones (46,47) indicated the presence of a 1.9 kb intron between position –11 and –12 relative to the start of translation. Additional studies have verified the organization of *CCR5* to include a short non-coding exon (43 bp), a 1.9 kb intron, followed by an exon that contains 11 bp of the 5′-UTR and the complete ORF (48,49). Using 5′-RACE, Mummidi *et al.* (50) described two additional 5′ untranslated exons and one additional intron. Exons 2 and 3 are not interrupted by an intron and, in agreement with the other reports (47–49), exon 4 (that containing the ORF) and exon 3 (exon 1 in the previous reports) are transcribed within several RNA isoforms of *CCR5*. There are two distinct promoters of *CCR5*, one of which lies upstream of exon 1 (P_u), and another that lies downstream in the region between intron 1 and exon 2 (P_d) (50). The identification and approximate location of P_d was concurrent among all studies characterizing the *CCR5* promoter region (48–51). The downstream promoter appears to be much stronger than the upstream promoter in monocytic and lymphocytic cell lines, as well as $CD4^+$ T cells (48–51). Sequence motifs similar to consensus sequences for a variety of transcription factors have been identified in the P_d promoter region and mutational analysis of several such sequences indicated their importance in transcription of *CCR5* (51). Thus, polymorphism in this region could potentially disrupt transcription factor binding motifs, accounting for some of the heterogeneity in CCR5 expression among individuals (52).

Several numbering systems for the CCR5 promoter region have been used throughout the literature. In order to standardize the nomenclature at this locus, a numbering system in which the first nucleotide of the translation start site is designated as position 1, and the nucleotide immediately upstream of this as position –1, was recently proposed at the CCR5 AIDS symposium held at the NCI-FCRDC, Frederick, MD, on April 30, 1999.

Linkage disequilibrium between *CCR5* promoter region and *CCR2-64I*

Genes encoding the CCR1-CCR5 molecules are clustered in the p21.3–p24 region of chromosome 3 (13,45,53). A human genomic DNA contig within this region (143068 bp) was sequenced as part of the Advanced Genome Sequence Analysis Course at Cold Spring Harbor Laboratory, NY (GenBank accession no. U95626; unpublished data). The contig shows that *CCR2* and *CCR5* are separated by only ~14 kb (Fig. 1), explaining in part the near complete linkage disequilibrium between the two genes (54). The variant *CCR2-64I* in the first transmembrane region of *CCR2* has been shown to be associated with relatively slow progression to AIDS (54,55) and this protection is genetically independent of that conferred by *CCR5-Δ32*. It seems unlikely that the *CCR2-64I* allele has a direct effect on AIDS progression because: (i) CCR2 is used as a co-receptor by only rare isolates of HIV-1 (5,6,56), and (ii) *CCR2-64I* encodes a product that binds chemokine ligand, and mediates both calcium mobilization signalling and R5 tropic HIV-1 infection as efficiently as wild-type CCR2 molecules (57). These observations along with the physical proximity of *CCR2* and *CCR5* have led to the speculation that *CCR2-64I* is simply tracking by linkage disequilibrium another variant of *CCR5* (55), in particular since all *CCR2-64I* bearing haplotypes are wild-type or normal with respect to the *CCR5* coding region (54). These observations provide further impetus for studying effects of variability in the regulatory region of *CCR5*.

The C→T transition variant at position 59653 [according to GenBank accession no. U95626, position 927 according to the numbering system of Mummidi *et al.* (50), and position *–1835* according to the new numbering system, which will be used throughout the remainder of this review] located in intron 2 of

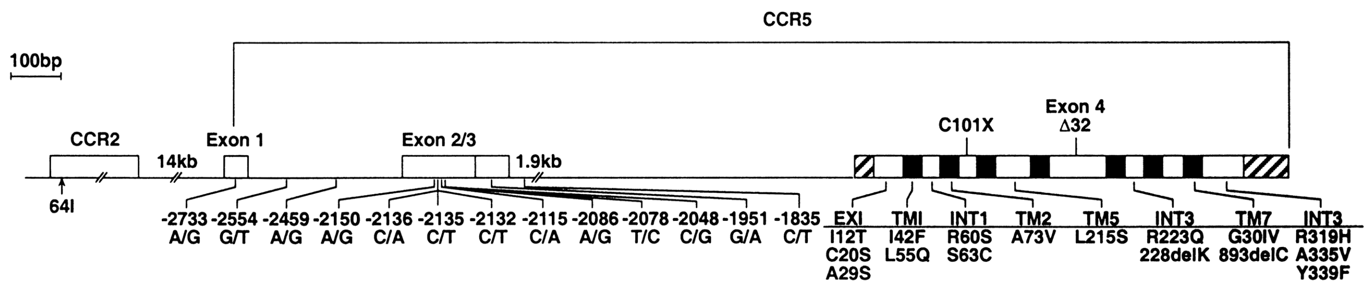

Figure 1. Map illustrating positions at which variations have been identified in the coding regions of *CCR2*, *CCR5* and 5′ upstream region of *CCR5*. The numbering system used designates the first nucleotide of the translation start site of *CCR5* as position 1, and the nucleotide immediately upstream of this as position –1.

Table 2. CCR5P alleles

New	–2733	–2554	–2459	–2150	–2136	–2135	–2132	–2115	–2086	–2078	–2048	–1951	–1835
Ref. 50[a]	29[b]	208[b]	303[b]	612	626	627[b]	630	647	676[b]	684[b]	714	811	927[b]
GenBank no. U95626[c]	58755	58934	59029	59338	59352	59353	59356	59373	59402	59410	59440	59537	59653
P1.1	A	G	A	A	C	C	C	C	A	T	C	G	C
P1.w2	G	–	–	n	n	–	n	n	–	n	n	n	–
P1.w3	–	–	–	n	n	–	n	n	–	n	n	n	T
P2.1	–	–	G	–	–	T	–	–	–	–	–	–	–
P3.1	–	T	G	–	–	T	T	–	–	–	–	–	–
P4.1	–	T	G	–	–	T	–	–	G	–	–	–	–
P5.1	–	–	G	G	–	T	–	–	–	–	–	–	–
P6.1	–	–	–	–	–	–	T	–	–	C	–	–	–
P7.1	–	–	–	–	A	–	–	–	–	–	–	–	–
P8.1	–	–	G	–	–	T	–	A	–	–	–	–	–
P9.1	–	–	G	–	–	T	–	–	–	–	–	A	–
P10.1	–	T	G	–	–	T	–	–	G	–	G	–	–
P11.w1	–	–	G	n	n	–	n	n	–	n	n	n	–

–, consensus to *P1*; n, sequence not known.
[a]According to Mummidi *et al.* (50).
[b]Identified by multiple labs.
[c]According to GenBank accession no. U95626.

CCR5 was found in 100% linkage disequilibrium with *CCR2-64I* (55). Although no data indicating a functional role in the control of *CCR5* expression has been observed for this variant (termed '*–1835T*' herein), it could explain the effect on AIDS progression seen in individuals with *CCR2-64I*. We have examined 984 individuals for both *CCR2-64I* and *–1835T (CCR5P-927T)* and found that all *CCR2-64I*-bearing haplotypes contained *–1835T*, but *–1835T* was also found infrequently on a haplotype carrying *CCR2*+ (16/230) (58). Nine of these were seroconverters and preliminary analysis of the nine hinted toward AIDS protection (relative hazard = 0.49 for AIDS-1993 definition), although significance was not reached. On the other hand, *–1835T* in the absence of *CCR2-64I* was associated with slightly accelerated disease progression in another study, although not significantly so (59). The question is not settled and further studies examining genetic associations and potential functional effects of *–1835T* must be addressed to determine whether it has a protective role in AIDS progression.

Additional polymorphism in the CCR5 promoter region

Several studies have addressed the possibility that polymorphism in the *CCR5* promoter region can account for some of the variability in progression to AIDS among HIV-1 infected individuals by altering levels of *CCR5* transcription (55,58–60). As mentioned in the previous section, two studies have addressed the effect of variation at position *–1835T* (previously 927) on progression to AIDS (55,59), but *–1835T* is in strong linkage disequilibrium with the *CCR2-64I* allele, so it is difficult to determine which variant (if either) is truly protective.

A number of additional variants in the 5′-UTR of *CCR5* have been identified (50,58,59) and seven of these distinguish four common promoter region alleles identified in Caucasian and African American samples (positions *–2733*, *–2554*, *–2459*, *–2135*, *–2132*, *–2086* and *–1835* in Fig. 1 and Table 2). The four alleles that we previously designated *CCR5P1*, *CCR5P2*, *CCR5P3* and *CCR5P4* (58) (Table 2) were observed at frequencies of 0.56, 0.085, 0.014 and 0.354, respectively, in Caucasians. (*CCR5P1-P4* were originally defined by the variant sites *–2554*, *–2135*, *–2132*, *–2086* and *–1835*, which completely distinguish the four common alleles defined by the seven positions listed above.) In order to determine the effects of these four alleles on progression to AIDS, it was necessary to identify haplotypes composed of the neighboring variants *CCR2*(*+/64I*) and *CCR5*(*+/*Δ*32*), since both alter rates of progression to AIDS (14,20–22,54,55). Six relatively common haplotypes composed of three loci, (i) the *CCR2* coding region variant *CCR2*(*+/64I*); (ii) the four promoter region alleles consisting of variants located from positions *–2733* through *–1835*; and (iii) the *CCR5* coding region variant *CCR5*(*+/*Δ*32*), were identified. The protective alleles *CCR2-64I* and *CCR5-*Δ*32* arose independently on two haplotypes containing the same promoter allele, *CCR5P1*. However, *CCR5P1* is found most frequently (f = 0.36) on a haplotype containing wild-type *CCR5* (*CCR5-+*) and wild-type *CCR2* (*CCR2-+*). Survival analyses in which individuals were partitioned by genotypes indicated presence of three distinct groups: (i) those homozygous for the haplotype *CCR2+-CCR5P1-CCR5+* who developed AIDS most rapidly; (ii) those with genotypes containing at least one copy of *CCR5-*Δ*32* or *CCR2-64I*, who had appreciably delayed onset of AIDS; and (iii) those with any other genotypic combination who developed AIDS at a rate intermediate between the susceptible and protected genotypes (58). Thus, homozygosity for *CCR5P1* may explain some of the variability in CCR5 expression known to occur among individuals who do not carry the *CCR5-*Δ*32* mutation (23).

Very similar results were observed for the A/G variant at position *–2459* (position 59029 of GenBank accession no. U95626) (60), which we now recognize as a component of *CCR5P1*. In this study, individuals who were homozygous for *–2459G* (found on *CCR5P2-P4*) progressed to AIDS 3–8 years more slowly than those who were homozygous for *–2459A* (found on *CCR5P1*). Of the 417 individuals typed for position *–2459* by McDermott *et al.* (60), 342 were also typed for *CCR5P* in the Martin *et al.* (58) study. Unfortunately, results from these two studies should not be considered as confirmatory since there was a high level of overlap in the patients tested by both groups and the variants tested were in very strong, if not complete linkage disequilibrium.

The simplest conclusion to be drawn from the described epidemiological data is that *CCR5P1* (including *–2459A*) has more efficient promoter activity than the other promoter alleles, leading to a relatively increased number of CCR5 receptors for HIV-1 on cell surfaces. Quantitative analysis of CCR5 on peripheral blood mononuclear cells from healthy volunteers representing two distinct genotypes, homozygosity for the haplotype *CCR2+-CCR5P1-CCR5+* or homozygosity for *CCR2+-CCR5P4-CCR5*, were studied for functional differences regarding *CCR5* expression. Cells representing each of these genotypes did not vary significantly in assays measuring (i) mean concentrations of CCR5; (ii) efficiency of promoting a luciferase reporter construct; and (iii) infectivity by R5 or R5/X4 strains of HIV-1 (58). It is possible that the effect of *CCR5P1* is too weak to be detected by the assay conditions used, for it is subtle enough only to be observed epidemiologically in *CCR2+-CCR5P1-CCR5* homozygotes. McDermott *et al.* (60), on the other hand, observed 45% lower promoter activity from a promoter allele containing the *–2459G* variant than that containing the *–2459A* variant. The *CCR5* promoter region segment used in their reporter-gene construct included all the variant sites described by Martin *et al.* (58), so it follows that the promoter allele used in their construct was *CCR5P1*. If so, this would suggest that there may indeed be a difference between the *CCR5P1* and other promoter region alleles.

More recently, we have used gel-shift assays to determine whether the variants at each of the five positions *–2554* (G/T), *–2459* (A/G), *–2135* (C/T), *–2086* (A/G) and *–1835* (C/T) differ in their ability to bind nuclear factors in T cell extracts (52). A clear difference in binding of one or more nuclear factors to oligonucleotides representing the two variants at position *–2554* (*–2554T* found on the *P4* allele and *–2554G* found on the *P1* allele) was observed. This provides enticing, albeit weak, support for the hypothesis that *CCR5* promoter region variation is responsible for the epidemiological effects observed in the AIDS cohorts.

SUMMARY

The central role of the CCR5 molecule in HIV-1 infection has led to a wealth of information over the past 3 years characterizing its functional and genetic properties. The mutant *CCR5-*Δ*32* allele has been shown to provide almost complete protection against HIV-1 infection in homozygous individuals. Heterozygous individuals also exhibit slower progression to AIDS after seroconversion, and protection from AIDS-related lymphoma. Additional variants, most of which are codon-altering, have also been identified, and functional analysis suggests that some of these variants may protect against HIV-1 infection as a result of severe alteration in the conformation of the molecule. The promoter region of the *CCR5* gene has been characterized by several groups, and it appears that polymorphisms in this region may have an effect on AIDS progression, possibly due to their effect on levels of CCR5 expression. These studies should prove useful not only in predicting outcome to HIV-1 infection, but also in developing novel therapeutic strategies.

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